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HUMANISED ANTIBODIES HAVING MODIFIED ALLOTYPIC DETERMINANTS

Abstract:

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<b>(54) Title:</b> HUMANISED ANTIBODIES HAVING MODIFIED ALLOTYPIC DETERMINANTS  <b>(57) Abstract</b>  The invention relates to molecules which have an amino acid sequence derivable from part or all of the constant region of an immunoglobulin heavy chain. The constant regions are of a particular isotype and have one or more allotypic determinants. The amino acid sequence is substantially homologous to the amino acid sequence of the constant region. However, it has been altered so that it is without at least one of said allotypic determinants by making its sequence the site for an allotypic determinant identical to the amino acid sequence from the corresponding position in another equivalent immunoglobulin constant region of a different isotype. The invention provides synthetic immunoglobulins with reduced allotypic differences as compared to a given wild-type immunoglobulin.		

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## Humanised antibodies having modified allotypic determinants

The present invention relates to binding molecules.  
In particular, it relates to recombinantly produced  
5 antibodies.

Owing to their high specificity for a given antigen,  
antibodies and particularly monoclonal antibodies  
(Kohler, G. and Milstein C., 1975 Nature 256:495)  
represented a significant technical break-through with  
10 important consequences scientifically, commercially and  
therapeutically.

Monoclonal antibodies are made by establishing an  
immortal cell line which is derived from a single  
immunoglobulin producing cell secreting one form of a  
15 biologically functional antibody molecule with a  
particular specificity.

Owing to their specificity, the therapeutic  
applications of monoclonal antibodies hold great promise  
for the treatment of a wide range of diseases (Clinical  
20 Applications of Monoclonal Antibodies, edited by E. S.  
Lennox. British Medical Bulletin 1984, publishers  
Churchill Livingstone). Antibodies are generally raised  
in animals, particularly rodents, and therefore the  
immunoglobulins produced bear characteristic features  
25 specific to that species. The repeated administration of  
these foreign rodent proteins for therapeutic purposes to

human patients can lead to harmful hypersensitivity reactions. In the main therefore, these rodent-derived monoclonal antibodies have limited therapeutic use. A further problem with these rodent derived antibodies, is  
5 that they are relatively ineffective at the depletion of cells in vivo, although the rat IgG2b antibody CAMPATH-1G is an exception to this rule.

Thus, there is a need for therapeutic antibodies which have characteristic features specific to human  
10 proteins. Unfortunately, immortal human antibody-producing cell lines are very difficult to establish and they give low yields of antibody (approximately 1 µg/ml). In contrast, equivalent rodent cell lines yield high amounts of antibody (approximately 100 µg/ml).  
15 Furthermore, where one wants to produce a human antibody with a particular specificity it is not practically or ethically feasible to immunise humans with an immunogen bearing the epitope of interest.

In part, this problem has been overcome in recent  
20 years by using the techniques of recombinant DNA technology to 'humanise' non-human antibodies. Structurally, the simplest antibody (IgG) comprises four polypeptide chains, two heavy (H) chains and two light (L) chains inter-connected by disulphide bonds (see  
25 figure 1). The light chains are of two types, either kappa or lambda. Each of the H and L chains has a region

of low sequence variability, the constant region (C) giving rise to allotypes and a region of high sequence variability, the variable region (V) giving rise to idiotypes. The antibody has a tail region (the Fc region) which comprises the C regions of the two H chains. The antibody also has two arms (the Fab region) each of which has a  $V_L$  and a  $V_H$  region associated with each other. It is this pair of V regions ( $V_L$  and  $V_H$ ) that differ from one antibody to another, and which together are responsible for recognising the antigen. In even more detail, each V region is made up from three complementarity determining regions (CDR) separated by four framework regions (FR). The CDRs are the most variable part of the variable regions, and they perform the critical antigen binding function. The CDR regions are derived from many potential germ line sequences via a complex process involving recombination, mutation and selection. It has been shown that the function of binding antigens can be performed by fragments of a whole antibody. Binding fragments are the Fv fragment which comprises the  $V_L$  and  $V_H$  of a single heavy chain variable domain ( $V_H$ ).

In creating "humanised" immunoglobulins, the Fc tail of a non-human antibody is exchanged for that of a human antibody. For a more complete humanisation, the FRs of the non-human antibody are exchanged for human FRs. This

process is carried out at the DNA level using recombinant techniques. However, these humanised immunoglobulins do not solve all the problems, because an immune response may still be mounted against the treatment antibody even when a patient is treated with a human antibody, as it may show certain sequence differences in the V (ie idiotypic differences) and C (ie allotypic differences) regions when compared with the patients own equivalent antibodies. This is a particular problem where the patient's immune system has already seen, and therefore been primed against, antibodies having these sequence differences (eg a patient may have received a prior blood transfusion which contained allotypically different immunoglobulins). A model system of injecting "mouseised human antibodies" into mice indicated that the allotype matching could critically affect the anti-idiotypic response (Bruggemann M., Winter G., Waldmann H., Neuberger M.S., (1989) J. Exp. Med. 170, 2153-2157).

The present applicants have realised that one way around this problem is to eliminate the allotypic variation from the constant region.

There are a range of different immunoglobulins IgG, IgM, IgA, IgD, IgE, known as isotypes, of which IgG is most commonly used therapeutically. It exists as isotypic sub-classes IgG1, IgG2, IgG3 and IgG4.

There are 24 recognised allotypes of human

immunoglobulin distributed between the different isotypes as follows:

5                   IgG1   x   4  
                  IgG2   x   1  
                  IgG3   x 13  
                  IgA2   x   2  
                  IgE     x   1  
                  Kappa x   3

10           The allotypes represent alternative amino acid substitutions found at discrete sites in the protein sequence. These different allotypic determinants are found in different combinations within given allelic forms of genes, but not all possible combinations which theoretically might exist are in practice observed.

15           For example, the four different allotypes of IgG1 can be seen (ie distinguished) by the immune system. These are G1m 1, 2, 3 and 17. Alternatively, combinations thereof, such as G1m (1, 17), can also be distinguished. The four different single allotypes are  
20 depicted in figure 2.

          Antisera can be raised in other non-human species which can see the alternative isoallotypes provided that the antibody is purified away from the other human isotypes. Such isoallotypes for which such an antisera  
25 exists have been called non-allotypes and given the designation for example, nG1m(1) which is the isoallotype



of G1m(1). Thus, although a human isoallotype should not be immunogenic in humans, it can still potentially be recognized in a different species.

Of the above mentioned different allotypes of IgG1, three common allelic forms of human IgG1 occur with different frequencies within different racial groups, namely G1m (3), G1m (1, 17), and G1m (1, 2, 17) based upon their reactivities with human antisera directed against the determinants G1m 1, 2, 3 and 17. At some point in the future, it is likely that a patient with an existing anti-allotype response to one or more of these determinants will need treatment with a humanised antibody. The obvious solution and one which has been proposed in a letter to the Journal Nature (Mage, R.G., Nature (1988) 333, 807-808), is to make all the different allelic forms of an antibody and to allotype match each patient for therapy. The present applicants have realised that commercially this is not a good proposal because of increased production costs and the need to process several reagents in parallel through the regulatory requirements. Additionally, each patient would have to be tested for the response to different allotypes.

Thus, the present applicants propose eliminating the allotypes altogether from each therapeutic antibody. The sequence of the human allotype of IgG1 G1m (1, 2, 17) is

shown aligned with sequences for the other human IgG, isotype sub-classes in figure 4 (a, b, c and d). It can be seen that the four isotypes are extremely homologous for the domains CH1, CH2 and CH3, and that the major isotypic differences are in the hinge region which varies in both, length and sequence between isotypes. The allotypic residues of IgG1 G1m (1, 2, 17) have been marked in figure 4. However, for the purposes of clarity the sequences around the allotypic sites G1m (1) (2) and (17) are shown below for each isotype.

Site (1)

	<u>355</u>	<u>356</u>	<u>357</u>	<u>358</u>	
	Arg	Asp or Glu	Glu	Leu or Met	IgG1
	Arg	Glu	Glu	Met	IgG2
15	Arg	Glu	Glu	Met	IgG3
	Gln	Glu	Glu	Met	IgG4

Thus, at site (1), IgG1 may exist as several allotypes depending on whether aspartic acid or glutamic acid at position 356, or leucine or methionine at position 358 are present.

Site 2

	<u>430</u>	<u>431</u>	<u>432</u>	
	Glu	Gly or Ala	Leu	IgG1
	Glu	Ala	Leu	IgG2
25	Glu	Ala	Leu	IgG3
	Glu	Ala	Leu	IgG4

Thus, at site (2), IgG1 may exist as either of two allotypes depending on whether glycine or alanine is present at position 431.

Site (17)/(3)

5 Sites (3) and (17) are alternative substitutions at the same site.

	<u>213</u>	<u>214</u>	<u>215</u>	
	Lys	Lys or Arg	Val	IgG1
	Lys	Thr	Val	IgG2
10	Lys	Arg	Val	IgG3
	Lys	Arg	Val	IgG4

Thus, at site (17)/(3), IgG1 may exist as either of two allotypes depending on whether lysine or arginine is present. The allotypes (17) and (3) cannot co-exist as  
 15 they represent alternative substitutions at the same position.

The alternative alleles of G1m (1) and (2) do not provoke a human allotype response because of the homology of these alleles with the other IgG sub-classes in this  
 20 region. These alleles are therefore called isoallotypes because they are only recognisable by xenoantisera (antisera from a different species) and only when the isotype is purified away from the other sub-classes.

Therefore, the present applicants propose the  
 25 creation of a new IgG1 allele by site-directed mutagenesis of the gene, for example, an existing

CAMPATH-1H monoclonal antibody gene described below, so that the new allele consists entirely of isoallotypic determinants. The preparation of IgG1 mutants according to the teaching provided by the present applicants is shown schematically in figure 3.

For G1m (1) and G1m (2), the changes comprise simple substitution by the alternative isoallotypic residues. However, in the case of G1m (17) the conversion of lysine to arginine would in some cases merely change the allotype to an allotype that is recognised by certain individuals as a G1m (3) allotype despite the fact that this residue is homologous with IgG3 and IgG4. This apparent contradiction is thought to be because this arginine is seen in a tertiary epitope in the context of the other IgG1 specific residues in close proximity in the CH1 domain or hinge region. This indicates that in addition to changing lysine, other residues in CH1 or the hinge will need to be changed in order to create a new isoallotype.

Although the above and ensuing description is specifically directed to IgG1 and in particular, the CAMPATH-1H monoclonal antibody, the same approach can be used to create isoallotypes of the other human isotypes such as IgG2, IgG3 and kappa.

Thus, the present invention provides a first binding molecule derivable from a second binding molecule;

which second binding molecule is an immunoglobulin, or a derivative, structural or functional analogue thereof, a member of a family of homologous molecules, and has one or more sites which are structurally  
5 distinctive from equivalent sites in the other family members;

wherein said first binding molecule is more closely homologous to the other family members than to said second binding molecule, at at least one of said one or  
10 more sites.

The first binding molecule may also be an immunoglobulin or a derivative, structural or functional analogue thereof. The one or more sites which are structurally distinctive from the equivalent sites in the  
15 other family members may be in the constant region giving rise to an allotypic difference. The first binding molecule may comprise entirely isoallotypic determinants.

The second binding molecule may be selected from the group consisting of IgG1, IgG2, IgG3, IgA2, IgE, kappa  
20 light chains or derivatives, structural or functional analogues thereof. Where the second binding molecule is IgG1, the allotypic differences may be present at one or more of sites (1) (2) (3) or (17) as described herein. Where the second binding molecule is IgG2, the allotypic  
25 difference may be present at site (23). Where the second binding molecule is IgG3, the allotypic differences may

be present at one or more of the sites (11) (5) (13) (14) (10) (6) (24) (21) (15) (16) (26) or (27). Where the second binding molecule is IgA2, the allotypic differences may be present at one or more of the sites (1) and (2). Where the second binding molecule is kappa light chain, the allotypic differences may be present at one or more of the sites (1) (2) or (3). The sites referred to above are well documented in the literature (see e.g. Eur. J. Immunol. 1976.6:599-601. Review of the notation for the allotypic and related marks of human immunoglobulins).

The present invention also provides pharmaceutical preparations comprising a first binding molecule as defined above or described herein together with one or more excipients. The pharmaceutical preparation may comprise a cocktail of said first binding molecules.

Also provided by the present invention are methods for making a first binding molecule as defined above or described herein.

These methods comprise the steps of: a) identifying in said second binding molecule, one or more sites which are structurally distinctive from the equivalent sites in the other family members; b) making said first binding molecule whereby it is more closely homologous to the other family members than to said second binding molecule at at least one of said one or more sites.

The first binding molecule may be made by providing a gene sequence encoding the second binding molecule and altering those parts of the gene sequence encoding said one or more sites. The gene sequence may be altered by site directed mutagenesis using oligonucleotide primers. The altered gene sequence may be incorporated into a cloning vector or expression vector. The expression vector may be used to transform a cell. The cell may be induced to express the altered gene sequence.

10 The present invention therefore provides cloning vectors and expression vectors incorporating the altered gene sequence. Also provided are cells transformed by expression vectors defined above. Also provided are cell cultures and products of cell cultures containing the first binding molecules. Also provided are recombinantly produced said first binding molecules.

20 Thus the present invention provides a molecule which comprises an amino acid sequence derivable from part or all of the constant region of an immunoglobulin heavy chain which constant regions are of a particular isotype and have one or more allotypic determinants

wherein said amino acid sequence is substantially homologous to the amino acid sequence of said constant region, but has been altered so that it is without at least one of said allotypic determinants, by making the amino acid residues at the site of an allotypic

determinant identical to the amino acid residues from the corresponding position in another equivalent immunoglobulin constant region of a different isotype.

The molecule may comprise an amino acid sequence  
5 derivable from part or all of a human immunoglobulin constant region.

The molecule may also comprise one or more polypeptides together with said amino acid sequence.

The polypeptide may comprise a functional biological  
10 domain. The domain may be such that it mediates any biological function. The functional biological domain may comprise a binding domain. The binding domain will have an ability to interact with another polypeptide. The interaction may be non-specific or specific.

15 The polypeptide, biological domain, binding domain and immunoglobulin-like binding domain may derive from the same source or a different source to the constant region.

The constant region may be from an immunoglobulin of  
20 the isotype IgG. The isotype subclass may be IgG1 and the molecule may no longer have one or more of the allotypic determinants 1,2,3 and 17. The isotype subclass may be IgG2 and the molecule may no longer have the allotypic determinant 23. The isotype subclass may  
25 be IgG3 and the molecule may no longer have one or more of the allotypic determinants 11,5,13,14,10,6,24,21,15,



16,26 and 27.

The constant region may be from an immunoglobulin of the isotype IgA2 and the molecule may no longer have either or both of the allotypic determinants 1 and 2.

5       The present invention also provides a pharmaceutical preparation which comprises a molecule as defined.

The present invention also provides a reagent which comprises a molecule as defined.

10       The present invention also provides a nucleotide sequence encoding a molecule as defined.

The present invention also provides cloning and expression vectors comprising a nucleotide sequence as delivered above.

15       The present invention also provides host cells comprising a cloning or expression vector as defined above.

The present invention also provides a method of preparing a molecule as defined above which comprises the steps of:

- 20   (a) identifying a constant region of an immunoglobulin heavy chain;
- (b) comparing the identified constant region with constant regions from immunoglobulin heavy chains of the same isotype to locate allotypic determinants in the
- 25   identified constant region;
- (c) obtaining the coding sequence for the identified

constant region having allotypic determinants;

(d) altering the coding sequence so that it codes for a molecule without at least one of said allotypic determinants and by making the amino acid residues at the site for an allotypic determinant identical to the amino acid residues from the corresponding position in an equivalent immunoglobulin constant region of an isotype different to that of said identified constant region;

(e) using said altered coding sequence in an expression system to produce a said molecule.

The present invention also provides a method of treating a patient which comprises administering a pharmaceutical preparation as defined above.

Of course, there are a number of different strategies which could be used in order to make the molecules with fewer allotypic determinants.

Genes encoding therapeutically useful antibodies are generally available in one of several different forms. They may be available as a cloned variable region DNA sequence with restriction sites at each end, suitable for recloning along with a chosen cloned constant region DNA sequence into a suitable expression vector. This is the strategy described herein for the constructs TF57-19, MTF121 and MTF123. Alternatively, they may be available as complete immunoglobulin DNA sequences including the V and C regions together, e.g. a cDNA clone of a complete

humanised or human antibody.

Whatever the form in which the cloned immunoglobulin gene is obtained, the next step is to predict the amino acid sequence of the constant region from the DNA sequence. The DNA sequence can be obtained using a variety of strategies familiar to molecular biologists. The predicted amino acid sequence would then be checked for the amino acids known to vary as allotypes. Any isoallotypes present within the sequence can be left unaltered. Any allotypes present can be mutated.

The next step, is to decide what amino acid sequence to mutate the allotype to, in order to imitate an isoallotype. This is done by lining up the sequence with the corresponding region of the other immunoglobulin isotypes. For all known allotypes, it has been found that one or more of the other isotypes have invariant sequences for the homologous region. One of these sequences can then be chosen to form the basis for the changes to be made in the allotype in question. Having predicted the new amino acid sequence for the constant region, it is necessary to alter the existing DNA clone or to create a new DNA clone which will encode this sequence. Again there are several strategies available to molecular biologists in order to achieve this. In the case of the example CAMPATH-1H constructs described

herein, the gamma-1 constant region was cloned in an M13TG131 single stranded phage vector. Mutagenic oligonucleotides were synthesised which were largely homologous to the single strand, but which contained base changes necessary to alter the codons for the critical amino acids. The mutagenesis was carried out using a commercial kit from Amersham International, High Wycombe, Bucks. Alternatively it would be possible to synthesise a complete artificial gene which encodes the predicted sequence.

Once mutated or newly synthesised, the gene is ready for expression. There are many different expression vectors available. Some of these are more suitable for expression in restricted cell types. Again it is within the standard technical expertise of one skilled in this field to choose and adapt expression vectors for this purpose. In the case of the CAMPATH-1H constructs described herein, modifications of the pSVgpt and pSVneo vectors have been used. These vectors have convenient cloning sites for the immunoglobulin variable and constant region, encoding DNA fragments adjacent to suitable promoter and enhancer sequences to allow expression in lymphoid cells. The vector allows the easy independent replacement of variable or constant region encoding DNA fragments. Thus, any suitable variable region can be subcloned into the vector, to give rise to

a new specificity, or the variable region can be kept and the constant region changed to give rise to a new isotype or allotype. Alternative vector systems are readily available.

- 5        Having removed allotypes from heavy chain constant regions by mutating them all to isoallotypes, it may still be desirable to consider the light chain effect in stimulating an immune response.

10        The most common kappa light chain allotype is Km(3) in the general population. Therefore it may be sufficient to utilise this common kappa light chain allotype, as relatively few members of the population would see it as foreign.

15        Alternatively there are no lambda light chain allotypes. Therefore they could be used in combination with the de-allotyped molecules derivable from heavy chain constant regions.

20        Where one utilises the kappa light chain, the allotype Km(1,2) could first be mutated to the allotype Km(1). The light chain allotype Km(1) is often only weakly recognized in combination with certain heavy chain classes and subclasses, and so may not cause a major problem in therapeutic use.

25        In order that the present invention is more fully understood embodiments will now be described in more detail, by way of example only, and not by way of

limitation. Reference will be made (and has already been made in the text above) to the following figures in which:

figure 1 illustrates the structure of an IgG  
5 antibody;

figure 2 shows the allotypes for the IgG1 antibody  
CAMPATH-1H;

figure 3 shows schematically the preparation of IgG1  
mutants;

10 figure 4 shows the IgG1 G1m (1,2,17) allotype  
sequence aligned to single allotypic examples of IgG2, 3  
and 4 (none of these other subclasses have allotypic  
residues which cover the same residues as for the IgG1  
allotypes);

15 figure 5 shows the M13TG131 cloning vector  
containing the human gamma-1 constant region, showing  
cloning sites and modified polylinker;

figure 6 shows the original Hu4vH HuG1 pSVgpt  
expression vector and its modified version;

20 figure 7 shows the result of an ELISA assay testing  
different dilutions of the antibodies of mutants 1, 2 and  
wild type CAMPATH-1H for IgG1 specificity;

figure 8 shows the result of an autologous  
complement mediated lysis test using human peripheral  
25 blood lymphocytes; and

figure 9 illustrates an antibody-dependent cell-

mediated cytotoxicity assay (ADCC) using CD3 activated interleukin-2 expanded human blastocytes cell effectors (E) and targets (T).

The starting antibody used for site-directed  
5 mutagenesis was CAMPATH-1H, a monoclonal antibody with a kappa light chain containing the human constant region sequence for IgG1 which carries the G1m (1, 17) allelic determinants. The whole IgG1 encoding region exists as approximately 2.3 kb HindIII-SphI restriction fragment  
10 cloned in an M13 vector. The M13TG131 cloning vector containing the human gamma-1 constant region showing cloning sites and modified polylinker is shown in figure 5.

The IgG1 encoding region is entered in the EMBL  
15 Sequence Database under the code number HS1GCC4. The accession number is AC J00228 (the printout from the database is provided herein as Appendix 1). This sequence is for the G1m (1, 17) allotype. It covers 2009 bases from the 5' HindIII site (A)AGCTT including all of  
20 the coding region. It does not however, include some of the 3' non-coding region up to the SphI site. The sequence provided by the EMBL Database is that of the upper strand of DNA. The CH1 domain starts at nucleotide 210 and ends at nucleotide 503. The mutagenic  
25 oligonucleotides M01 and M04 hybridise to nucleotides 486 to 510. The hinge region starts at nucleotide 892 and

ends at nucleotide 936. The CH2 domain starts at nucleotide 1481 and ends at nucleotide 1803. The mutagenic oligonucleotide MO2 hybridises to nucleotides 1515 to 1543. The essential signal for the poly A tail  
5 is provided by nucleotides 1902 to 1908.

In M13TG131, the IgG1 coding region exists as a 2260 nucleotide fragment, of which the final 251 nucleotides are non-coding and therefore, inessential. Therefore, an embodiment of the invention could be carried out using  
10 the sequence information provided by the EMBL Sequence Database. It should be noted however, that the SphI restriction site referred to above, is present in the 3' end inessential non-coding region. Therefore, if the sequence data as provided by the EMBL database were being  
15 used, alternative restriction sites would have to be utilised.

Using site-directed mutagenesis, (carried out using protocols and reagents as supplied in kit form, Amersham code RPN. 1523, Amersham International Plc, Amersham, UK)  
20 the sequence corresponding to the Glm (1) allele was converted to the corresponding sequence found in the other sub-classes for IgG (Asp Glu Leu to Glu Glu Met at positions 356-358 in the CH3 domain).

The mutagenic oligonucleotides used were:

- 25 a) MO1 (to convert Glm (17) to Glm (3))  
5' CTC TCA CCA ACT CTC TTG TCC ACC T 3';



- b) MO2 (to convert G1m (1) to its isoallotype nG1m (1))  
5' GGT TCT TGG TCA TCT CCT CCC GGG ATG GG 3'; and
- c) MO4 (to eliminate G1m(3) by changing Lys to Thr in  
the CH1 region)

5 5' CTC TCA CCA ACA GTC TTG TCC ACC T 3'.

The oligonucleotides were synthesised and then purified  
using an automated synthesizer and oligo purification  
columns supplied by Applied Biosystems (Applied  
Biosystems, 850 Lincoln Drive, Foster City, California,  
10 94404 USA) following the manufacturers recommended  
protocols. Mutations were checked by Sanger Dideoxy  
sequencing (Sanger, F.S., Nicklen, S., and Coulson, A.R.,  
(1977) Proc. Natl. Acad. Sci., USA, 74, 5463) using  
standard protocols and kits. As this newly formed  
15 allotype sequence is found in all humans, there should be  
no immunological response to this alternative form of G1m  
(1). Additionally and similarly, the lysine residue  
responsible for the G1m (17) allotypic determinant was  
converted to an arginine residue corresponding to the G1m  
20 allele (Lys 214-Arg; mutant 1).

The gene for this new constant region of mutant 1  
carrying these three changes has been sequenced,  
incorporated into an expression vector containing the  
CAMPATH-1H V-region and expressed together with the  
25 CAMPATH-1H light chain which had been introduced by co-  
transfection.

A further mutant has been made by replacing the critical arginine residue associated with G1m (3) of mutant 1 with a threonine residue, to produce a heavy chain which is the equivalent of IgG2 and which should fail to react with both anti-G1m (17) and anti-G1m (3) antisera (mutant 2).

Mutant 2 has also been sequenced, re-cloned in an expression vector containing the CAMPATH-1H light chain.

The supernatants of growing cultures containing either of the two mutants were subsequently assayed for the expression of a human IgG1 kappa product.

The mutations were introduced with the oligonucleotides listed above. The modified Hu4vHG1pSVgpt vector shown in figure 6 was used to simplify the subcloning of all the new mutants into the expression vector, owing to the possibility of use of two different "sticky ends" Bam HI and NotI. The expression vectors and V<sub>H</sub> region sequences and expression, along with the light chains, in YO rat plasmacytoma cells are all as described in Riechmann L., Clark, M.R. Waldman H., Winter G. (1988) Nature 332, 323-327.

From the positive cultures, the producers of the largest amount of the IgG1 product were selected to obtain purified antibody for biological assays to determine their allotypes and biological effector functions.

Example 1

An Enzyme-linked Immuno Sorbent Assay (ELISA) was performed to verify that an IgG1 type antibody was produced by the mutants. This was tested with microtiter  
5 plates coated with anti-CAMPATH-idiotypic antibody (YID 13.9). Wild type CAMPATH-1H antibody served as control. The bound antibody was detected with biotin-labelled anti-human kappa reagents or anti-human IgG reagent (monoclonals NH3/41 and NH3/130 respectively although  
10 other suitable reagents are commonly available) and subsequent development with streptavidin horseradish peroxidase. Figure 7 illustrates the results obtained for:

TF 57-19 ("wild type" CAMPATH-1H antibody, 0)  
15 MTF 121 (mutant 1,  $\Delta$ )  
MTF 123 (mutant 2,  $\square$ )

and the wild type CAMPATH-1H ( $\nabla$ ) in a known amount as standard. The concentrations had been estimated, and the starting dilutions adjusted to 50  $\mu$ g/ml in PBS/10 mg/ml  
20 BSA. The starting dilution was used to prepare 8 two-fold dilutions.

The slope of the graph shows clearly that the CAMPATH-idiotypic antibodies recognises mutants 1 and 2 to an extent equivalent to that of the wild type CAMPATH-1H,  
25 and that all three antibodies tested are present in similar concentrations as the standard.

Example 2

The mutants' capability of autologous complement mediated lysis of human peripheral blood lymphocytes was tested.

5 Human peripheral blood mononuclear cells from a healthy donor were isolated from 60 ml defibrinated blood on a Lymphoprep\* gradient (Nyeggard & Co., AS, Oslo, Norway). The cell pellet was washed in IMDM (Iscove's Modification of Dulbecco's Medium, Flow Laboratories,  
10 Scotland), and the cells were labelled with  $^{51}\text{Cr}$ . The starting dilution of antibodies used in the test was 50  $\mu\text{g/ml}$  in PBS, 10  $\mu\text{g/ml}$  BSA (dilution 1). Dilution 1 was further diluted 8 times two-fold to a final dilution of 1/128. Wild type antibody diluted in the same manner was  
15 used as a control.

The result is illustrated in figure 8. As can be seen, both antibody mutants show a very similar result in lysing the blood mononuclear cells as the wild type. The efficiency of the mutants is almost identical.

20 Example 3

Experiments were conducted to investigate whether or not, the mutant antibodies were capable of antibody-dependent cell-mediated cytotoxicity (ADCC) using CD3 activated interleukin-2 expanded human blastocytes as  
25 effectors (E) and targets (T). Cells were generated and used as both effectors and targets essentially as

described in Riechmann L., Clark M.R., Waldmann H., Winter G., 1988, Nature 322, 323-327.

Preparation of Target Cells (T)

5 ml of blastocytes ( $3 \times 10^6$  cells) were labelled with  $^{51}\text{Cr}$  for 1 h. After 1 h the cells were washed and transferred in 6 equal aliquots in 100  $\mu\text{l}$  IMDM 1% BSA, to 6 x 10 ml tubes containing 100  $\mu\text{l}$  of the antibodies of mutants 1 and 2, and the control. The tubes were incubated for 1.5 h at room temperature. The cells were then washed with 10 ml IMDM 1% BSA and resuspended in 1.5 ml IMDM 1% BSA.

Preparation of Effector Cells (E)

Unlabelled blastocytes ( $2 \times 10^6$ ) were diluted 100:1 and 30:1 in IMDM 1% BSA medium. The ratios 100:1 and 30:1 refer to the final absolute ratios of effectors to  $^{51}\text{Cr}$  labelled targets in the assay. Assays were performed in microtitre plates with a total volume of 200  $\mu\text{l}$  per assay well. Thus 100  $\mu\text{l}$  of targets at a concentration of  $2 \times 10^4$  were put in each well ie  $2 \times 10^3$  total cells. For E:T of 100:1, 100  $\mu\text{l}$  of effectors at  $2 \times 10^6$  were plated per well ie  $2 \times 10^5$ . For E:T of 30:1 100  $\mu\text{l}$  of effectors at  $6 \times 10^5$  were put into each well ie  $6 \times 10^4$  total cells.

The efficiency percentage of specific  $^{51}\text{Cr}$  release was calculated as follows:

$$\% \text{ specific } ^{51}\text{Cr} \text{ release} =$$

(test release cpm - spontaneous (cpm) x 100

(total cpm - spontaneous cpm)

cpm = radioactive counts per minute as measured on a counter.

5        The result is shown in figure 9. The figure shows that all of the antibodies tested released chromium. Wild type TF 57-19 and mutant 2 (MTF 123) released at about equal levels, whereas mutant 1 (MTF 121) shows a slightly higher release.

10       These results clearly show that the mutants have biological activity comparable to the wild type CAMPATH-1H antibody.

Example 4

15       The antibodies were tested in an assay specific for their Glm (3) allotypes reactivity using a monoclonal reagent from Oxoid (WHO/IVISS recognised agent, Study Code No HP 6027). These tests were performed in replicates of two.

20       Microtiter plates were coated with the anti-CAMPATH idiotypic YID 13.9.4 antibody captive, and divided into three arrays of 4 x 4 wells. Into each of the three arrays, 4 x 5 fold dilutions of the antibody TF 57-19, MTF 121 and MTF 123 (50 µg/ml) in PBS 1% BSA and a control solution of PBS/BSA each were added.

25       After an incubation of 45 minutes at room temperature, the antibody solution was removed, and

(i) to the first array was added a 1:500 dilution of biotin-labelled anti-Glm (3);

(ii) to the second array was added a 1:100 dilution of biotin-labelled antibody (NH3/41) specific  
5 for the kappa light chain; and

(iii) to the third array was added a 1:1000 dilution of biotin-labelled antibody (NH3/130) specific for human IgG1.

The microtiter plate was developed with streptavidin  
10 horseradish peroxidase.

The result is illustrated in Table 1. The numbers in the results represent the optical density (O.D) as measured in an ELISA plate reader multiplied by 100 ie 12 represents an O.D of 0.12 and 70 an O.D of 0.70.

15 The result clearly shows, that samples 1-3 all react with the antibodies specific for IgG1 (see also Example 1 above) and the kappa light chains. The control is negative. However, in the assay for Glm (3) specificity, only MTF 121 (mutant 1) shows reactivity, whereas the  
20 wild type TF 57-19, MTF 123 (mutant 2) and the PBS/BSA control did not show any response.

This result illustrates clearly that the elimination of sites recognised in the allotype response by site-directed mutagenesis of these sites can overcome the  
25 problems of allotypic immuno-reactions. Although the examples refer to the mutagenesis of IgG1 only, it will

be clear to the person skilled in the art that other immunoglobulin isotypes can be similarly modified.

Example 5

The antibodies were tested in a conventional  
5 allotyping experiment using inhibition of red cell agglutination. The experiment was carried out using reagents supplied by the Central Laboratory of the Netherlands Red Cross, Blood Transfusion Service (PO Box 9190, 1006 AD Amsterdam, Netherlands).

10 Human blood group O Rhesus D red cells were washed and then aliquots separately labelled as described below with one of the following three relevant anti-RhD human sera having antibodies of known allotype.

- (1) anti-D Glm(az) = Glm (1,17)
- 15 (2) anti-D Glm(x) = Glm (2)
- (3) anti-D Glm(f) = Glm (3)

Coating of Red Cells with Anti-Rh Antibodies

One volume of packed washed red blood cells were incubated with 4 volumes anti-Rh serum and 4 volumes  
20 (phosphate) buffered saline (PBS) at 37°C during 60 minutes. Every 15 minutes the cells were mixed by shaking.

After incubation the coated cells were washed four times with PBS and stored at 4°C in preservation fluid  
25 (although coated red blood cells can be stored at 4°C in PBS for one week).



These coated red blood cells were then agglutinated with four antisera to the IgG1 allotypes as follows using the recommended dilution for each antiserum.

- (1) anti-Glm(z) = anti-Glm(17) 1 in 30 dilution
- 5 (2) anti-Glm(a) = anti-Glm(1) 1 in 30 dilution
- (3) anti-Glm(x) = anti-Glm(2) 1 in 20 dilution
- (4) anti-Glm(f) = anti-Glm(3) 1 in 30 dilution

The wild-type CAMPATH-1H TF57-19 or the different CAMPATH-1H constructs (MTF 121, MTF 123) with the altered  
 10 gamma-1 constant regions were then tested for their abilities to inhibit the agglutination of the red cells by the above antisera. The inhibiting antibodies were tried at concentrations of 0.5mg/ml, 0.25mg/ml and 0.125mg/ml in phosphate buffered saline containing 5%  
 15 foetal bovine serum. Control sera containing IgG1 of allotype Glm(zax) or Glm(f) [Glm(1,2,17) or Glm(3)] were also included in the experiment and were used at dilutions of 1 in 10,20 and 40. Where it occurred the inhibition was most easily seen for the CAMPATH-1H  
 20 antibodies at the 0.5mg/ml concentration and it was much weaker for 0.25mg/ml and no inhibition was seen at 0.125mg/ml. The control sera inhibited at all three dilutions tested. The results for the highest concentration are shown below.

25

Allotype	CAMPATH-1H constructs	Control sera
----------	-----------------------	--------------

31

	TF57-19	MTF121	MTF123	G1m(1,2,17)	G1m(3)
G1m(1)	+	-	-	+	-
G1m(2)	-	-	-	+	-
G1m(3)	-	+	-	-	+
5 G1m(17)	+	-	-	+	-

The results are therefore consistent with the original wild type CAMPATH-1H antibody TF57-19 having allotype G1m(1,17). The new mutant MTF121 type as  
10 allotype G1m(3) whilst the mutant MTF123 fails to allotype for any of the IgG1 allotype markers G1m(1,2,3,17) i.e. it appears not to have an IgG1 allotype.

The skilled man will be able to use the binding  
15 molecules hereby provided to make pharmaceuticals according to standard techniques. Similarly the pharmaceuticals can be used in accordance with standard practices.

32

Table 1

Type Specific Antibody	anti Glm(3)				anti kappa (NH3/41)				anti IgG 1 (NH3/130)			
	10	2	0.4	0.08	10	2	0.4	0.08	10	2	0.4	0.08
1) TF 57-19 (Wildtype)	12/10	13/12	13/12	13/11	52/59	52/53	45/47	27/30	61/66	65/66	54/53	28/23
2) MTF 121 (Mutant 1)	80/75	69/69	64/65	53/44	59/53	54/52	48/41	28/20	71/68	71/69	55/52	26/23
3) MTF 123 (Mutant 2)	17/16	15/17	16/16	16/17	56/58	55/60	50/55	36/36	67/73	66/70	57/63	31/35
4) PBS/BSA	15/16	15/16	15/18	15/19	15/16	17/17	18/18	15/18	15/17	15/16	15/15	15/16

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APPENDIX 1 - Sheet (a)

HSIGCC4 2009 bases

Human ig germline g-e-a region a: gamma-1 constant region

ID HSIGCC4 standard; DNA; PRI; 2009 BP.

AC J00228;

DT 23-APR-1990 (reference update)

DT 18-JUL-1985 (incorporated)

DE Human ig germline g-e-a region a: gamma-1 constant  
DE region

KW constant region; gamma-immunoglobulin; germ line;  
KW hinge exon; immunoglobulin; immunoglobulin heavy  
KW chain.

OS Homo sapiens (human)

OC Eukaryota; Metazoa; Chordata; Vertebrata; Tetrapoda;

OC Mammalia; Eutheria; Primates.

RN [1] (bases 1-2009)

RA Ellison J.W., Berson B.J., Hood L.E.;

RT "The nucleotide sequence of a human immunoglobulin  
RT c-gamma-1 gene";

RL Nucleic Acids Res. 10:4071-4079(1982).

RN [2] (bases 469-1070, 1465-1821)

RA Takahashi N., Ueda S., Obata M., Nikaido T.,

RA Nakai S., Honjo T.;

RT "Structure of human immunoglobulin gamma genes:

RT Implications for evolution of a gene family";

RL Cell 29:671-679(1982).

CC [1] and [2] report that nucleotide divergence among  
CC the four gamma genes is much greater in the hinge  
CC regions than anywhere else. [2] also reports the  
CC hinge regions of gamma-2, gamma-3, gamma-4, a gamma  
CC pseudogene, and the 5' flanking, ch2, and ch3  
CC domains of the gamma genes.

CC this entry is part of a multigene region (region a)  
CC containing the gamma-3, gamma-1, pseudo-epsilon, and  
CC alpha-1 genes. see segment 1 for more comments.

Key Location/Qualifiers

FT CDS 210..503  
FT /note="Ig gamma-1 heavy chain

APPENDIX 1 - cont. Sheet (b)

FT		c-region ch1 domain (aa at 212)"
FT	conflict	563..563
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FT		/note="T in [1]; c in [2]"
FT	conflict	593..593
FT		/citation=([1],[2])
FT		/note="C in [1]; t in [2]"
FT	conflict	614..614
FT		/citation=([1],[2])
FT		/note="G in [1]; a in [2]"
FT	conflict	633..633
FT		/citation=([1],[2])
FT		/note="G in [1]; gg in [2]"
FT	conflict	643..643
FT		/citation=([1],[2])
FT		/note="G in [1]; a in [2]"
FT	conflict	654..654
FT		/citation=([1],[2])
FT		/note="G in [1]; a in [2]"
FT	conflict	684..684
FT		/citation=([1],[2])
FT		/note="C in [1]; cc in [2]"
FT	conflict	692..692
FT		/citation=([1],[2])
FT		/note="G in [1]; a in [2]"
FT	conflict	765..766
FT		/citation=([1],[2])
FT		/note="Aa in [1]; a in [2]"
FT	CDS	892..936
FT		/note="Ig gamma-1 heavy chain
FT		c-region hinge"
FT	CDS	1055..1384
FT		/note="Ig gamma-1 heavy chain
FT		c-region ch2 domain"
FT	conflict	1475..1475
FT		/citation=([1],[2])
FT		/note="C in [1]; cc in [2]"
FT	CDS	1481..1803
FT		/note="Ig gamma-1 heavy chain
FT		c-region ch3 domain"
FT	conflict	1578..1578
FT		/citation=([1],[2])
FT		/note="T in [1]; c in [2]"
SQ	Sequence	2009 BP; 418 A; 698 C; 566 G; 327 T; 0
SQ	Other;	

## APPENDIX 1 - cont. Sheet (c)

1	AGCTTTCTGG	20	GGCAGGCCAG	30	GCCTGACCTT	40	GGCTTTGGGG	50	CAGGGAGGGG	60	GCTAAGGTGA
61	GGCAGGTGGC		GCCAGCAGGT		GCACACCCAA		TGCCCATAGAG		CCCAGACACT		GGACGCTGAA
121	CCTCGCGGAC		AGTTAAGAAC		CCAGGGGCGCT		CTGCGGCTGG		GCCCAGCTCT		GTCCCCACACC
181	GCGGTACAT		GGCACCCACT		CTCTTGACAG		CTCCACCAAG		GGCCCATCGG		TCTTCCCCCT
241	GGCACCCCTCC		TCCAAGAGCA		CCTCTGGGGG		CACAGCGGCC		CTGGGCTGCC		TGGTCAAGGA
310		320		330		340		350		360	
301	CTACTTCCCC		GAACCGGTGA		CGGTGTCGTG		GAACCTAGGC		GCCCTGACCA		GCGGCGGTGCA
361	CACCTTCCCG		GCTGTCCCTAC		AGTCCTCAGG		ACTCTACTCC		CTCAGCAGCG		TGGTGACCGT
421	GCCCTCCAGC		AGCTTGGGCA		CCCAGACCTA		CATCTGCAAC		GTGAATCACA		AGCCCAGCAA
481	CACCAAGGTG		GACAAGAAAG		TTGGTGAGAG		GCCAGCACAG		GGAGGGAGGG		TGTCTGCTGG
541	AAGCAGGCTC		AGCGCTCCTG		CCTGGACGCA		TCCC GGCTAT		GCAGCCCCAG		TCCAGGGCAG
610		620		630		640		650		660	
601	CAAGGCAGGC		CCCGTCTGCC		TCTTCACCCG		GAGCCTCTGC		CCGCCCCACT		CATGCTCAGG
661	GAGAGGGTCT		TCTGGCTTTT		TCCAGGCTC		TGGGCAGGCA		CAGGCTAGGT		GCCCCTAACC
721	CAGGCCCTGC		ACACAAAGGG		GCAGGTGCTG		GGCTCAGACC		TGCCAAGAGC		CATATCCGGG
781	AGGACCCCTGC		CCCTGACCTA		AGCCCAACCC		AAAGGCCAAA		CTCTCCACTC		CCTCAGCTCG
841	GACACCTTCT		CTCCTCCCCAG		ATTCCAGTAA		CTCCCAATCT		TCTCTCTGCA		GAGCCCCAAT
910		920		930		940		950		960	
901	CTTGTGACAA		AACTCACACA		TGCCCAACCGT		GCCCAGGTAA		GCCAGCCCCAG		GCCTCGCCCT
961	CCAGCTCAAG		GCGGGACAGG		TGCCCTAGAG		TAGCCTGCAT		CCAGGGACAG		GCCCCAGCCG
1021	GGTGCTGACA		CGTCCACCTC		CATCTCTTCC		TCAGCACCTG		AACCTCCTGG		GGGACCCGTC
1081	GTCTTCCTCT		TCCCCCCTAA		ACCCAAGGAC		ACCTCATGA		TCTCCCGGAC		CCCTGAGGTC
1141	ACATGCGTGG		TGGTGGACGT		GAGCCACGAA		GACCTGAGG		TCAAGTTCAA		CTGGTACGTG

APPENDIX 1 - cont. Sheet (d)

1201	1210	1220	1230	1240	1250	1260
GACGGCGTGG	AGGTGCATAA	TGCCAAGACA	AAGCCGCGGG	AGGAGCAGTA	CAACAGCAGG	
1261	TACCGGGTGG	TCAGCGTCCT	CACCGTCCTG	CACCAGGACT	GGCTGAATGG	CAAGGAGTAC
1321	AAGTGCAAGG	TCTCCAACAA	AGCCCTCCCA	GCCCCCATCG	AGAAAACCAT	CTCCAAAGCC
1381	AAAGGTGGGA	CCCGTGGGGT	GCGAGGGCCA	CATGGACAGA	GGCCGGCTCG	GCCCA'CCCTC
1441	TGCCCTGAGA	GTGACCGCTG	TACCAACCTC	TGTCCTACAG	GGCAGCCCCG	AGAACCACAG
1501	1510	1520	1530	1540	1550	1560
GTGTACACCC	TGCCCCCATC	CCGGGATGAG	CTGACCAAGA	ACCAGGTCAG	CCTGACCTGC	
1561	CTGGTCAAAG	GCTTCTATCC	CAGCGACATC	GCCGTGGAGT	GGGAGAGCAA	TGGGCAGCCG
1621	GAGAACAACT	ACAAGACCAC	GCCTCCCGTG	CTGGACTCCG	ACGGCTCCTT	CTTCCTCTAC
1681	AGCAAGCTCA	CCGTGGACAA	GAGCAGGTGG	CAGCAGGGGA	ACGTCTTCTC	ATGCTCCGTG
1741	ATGCATGAGG	CTCTGCACAA	CCACTACACG	CAGAAGAGCC	TCTCCCTGTC	TCCGGGTAAA
1801	1810	1820	1830	1840	1850	1860
TGAGTGGGAC	GGCCGGGCAAG	CCCCGCTCCC	CGGGCTCTCG	CGGTGCGCAG	AGGATGCTTG	
1861	GCACGTACCC	CCTGTACATA	CTTCCCGGGC	GCCCAGCATG	GAAATAAAGC	ACCCAGCGCT
1921	GCCCTGGGCC	CCTGCGAGAC	TGTGATGGTT	CTTCCACCGG	GTCAGGGCCGA	GTCTGAGGCC
1981	TGAGTGGCAT	GAGGGAGGCA	GAGCGGGTC			

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CLAIMS

1. A molecule which comprises an amino acid sequence derivable from part or all of the constant region of an immunoglobulin heavy chain which constant regions are of a particular isotype and have one or more allotypic determinants

wherein said amino acid sequence is substantially homologous to the amino acid sequence of said constant region, but has been altered so that it is without at least one of said allotypic determinants, by making the amino acid residues at the site of an allotypic determinant identical to the amino acid residues from the corresponding position in another equivalent immunoglobulin constant region of a different isotype.

2. A molecule according to claim 1 which comprises an amino acid sequence derivable from part or all of a human immunoglobulin constant region.

3. A molecule according to claim 1 or claim 2 which comprises one or more polypeptides together with said amino acid sequence.

4. A molecule according to claim 3 wherein the polypeptide comprises a functional biological domain.



5. A molecule according to claim 4 wherein the functional biological domain comprises a binding domain.

5 6. A molecule according to claim 5 wherein the binding domain is an immunoglobulin-like binding domain.

7. A molecule according to claim 6 in which said immunoglobulin-like binding domain and said amino acid  
10 sequence are derivable from the same or different sources.

8. A molecule according to any one of claims 1 to 7 wherein the constant region is from an immunoglobulin of  
15 the isotype IgG.

9. A molecule according to claim 8 wherein the isotype subclass is IgG1 and the molecule no longer has one or more of the allotypic determinants 1,2,3 and 17.

20 10. A molecule according to claim 8 wherein the isotype subclass is IgG2 and the molecule no longer has the allotypic determinant 23.

25 11. A molecule according to claim 8 wherein the isotype subclass is IgG3 and the molecule no longer has one or

more of the allotypic determinants 11,5,13,14,10,6,24,21,  
15,16,26 and 27.

5 12. A molecule according to any one of claims 1 to 7  
wherein the constant region is from an immunoglobulin of  
the isotype IgA2 and the molecule no longer has either or  
both of the allotypic determinants 1 and 2.

10 13. A pharmaceutical preparation which comprises a  
molecule according to any one of claims 1 to 12.

14. A reagent which comprises a molecule according to  
any one of claims 1 to 12.

15 15. A nucleotide sequence encoding a molecule according  
to any one of claims 1 to 12.

16. A cloning or expression vector comprising a  
nucleotide sequence according to claim 15.

20

17. A host cell comprising a cloning or expression  
vector according to claim 16.

25 18. A method of preparing a molecule according to any  
one of claims 1 to 12 which comprises the steps of:

(a) identifying a constant region of an immunoglobulin

heavy chain;

(b) comparing the identified constant region with constant regions from immunoglobulin heavy chains of the same isotype to locate allotypic determinants in the identified constant region;

(c) obtaining the coding sequence for the identified constant region having allotypic determinants;

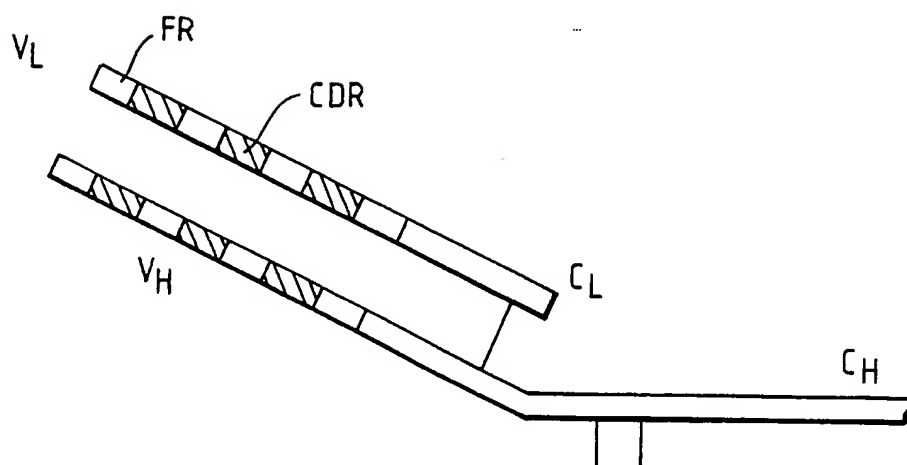
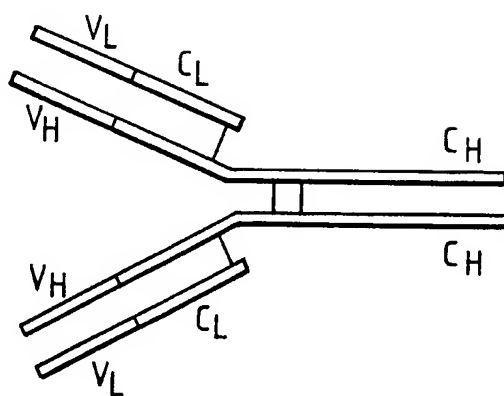
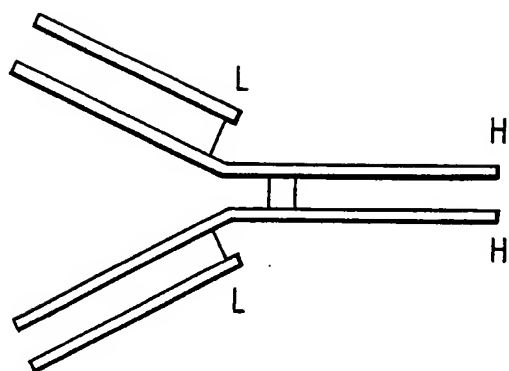
(d) altering the coding sequence so that it codes for a molecule without at least one of said allotypic determinants and by making the amino acid residues at the site for an allotypic determinant identical to the amino acid residues from the corresponding position in an equivalent immunoglobulin constant region of an isotype different to that of said identified constant region;

(e) using said altered coding sequence in an expression system to produce a said molecule.

19. A method of treating a patient which comprises administering a pharmaceutical preparation according to claim 13.

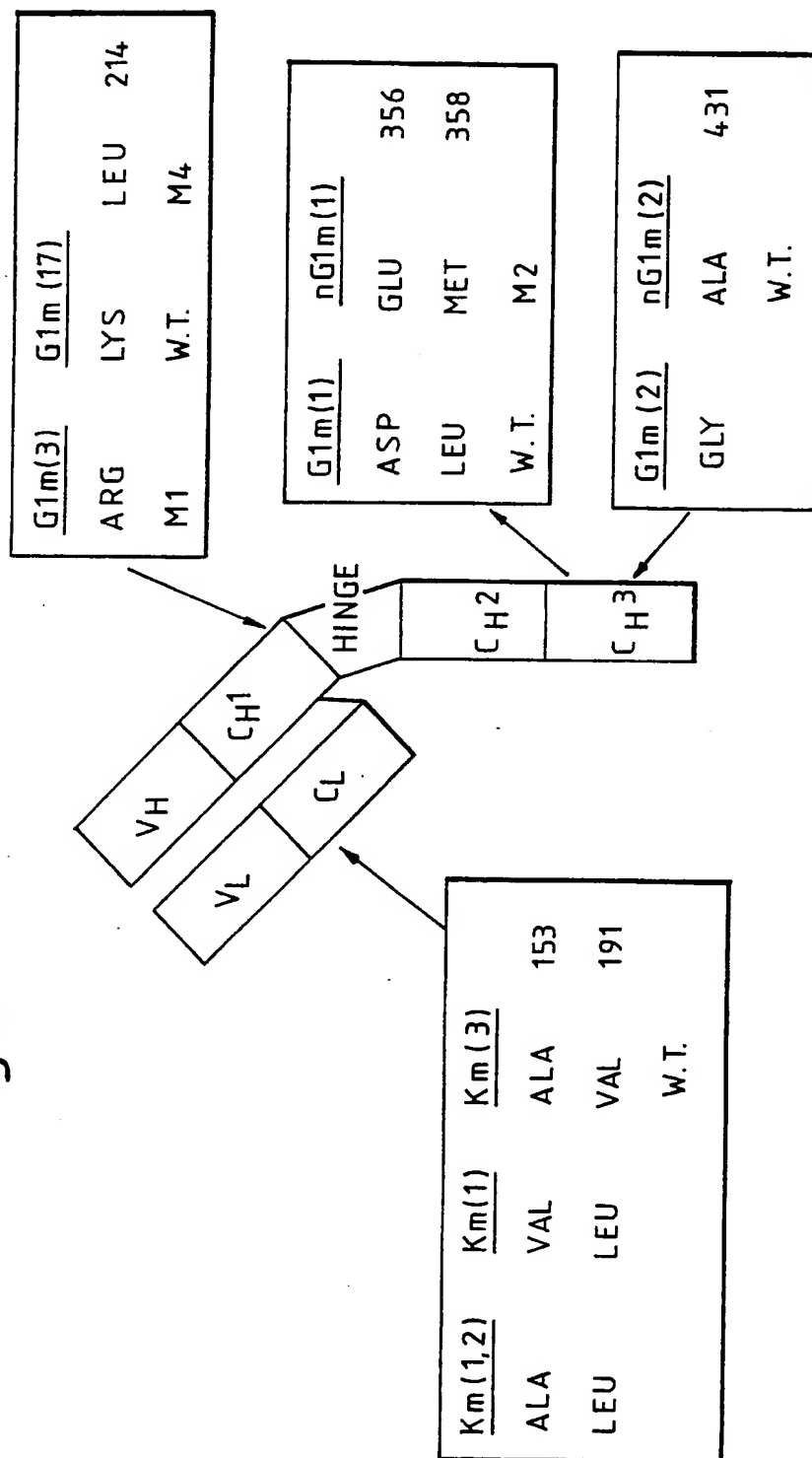
1/11

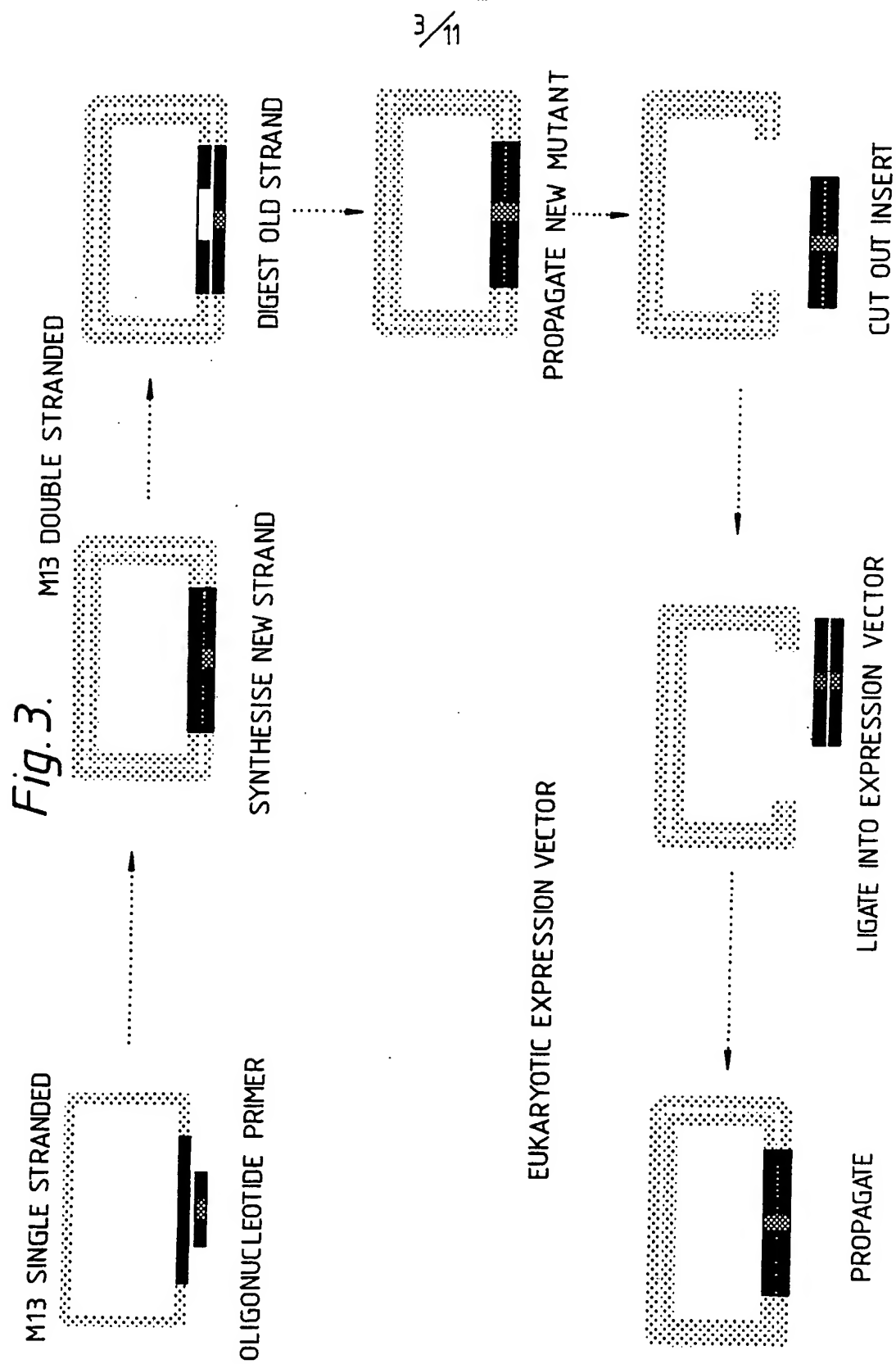
Fig. 1.



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Fig. 2.





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Fig. 4a.

Human immunoglobulin sequences CH1 region

AlaSerThrLysGlyProSerValPheProLeuAlaProSerSerLysSerThrSerGly	IgG1
- - - - - - - - - - - - - - - Arg - - - Glx	IgG2
- - - - - - - - - - - - - - - Cys - Arg - - - -	IgG3
- - - - - - - - - - - - - - - Cys - Arg - - - - Glu	IgG4

GlyThrAlaAlaLeuGlyCysLeuValLysAspTyrPheProGluProValThrValSer	IgG1
Ser -	IgG2
- -	IgG3
Ser -	IgG4

TrpAsnSerGlyAlaLeuThrSerGlyValHisThrPheProAlaValLeuGlnSerSer	IgG1
- -	IgG2
- -	IgG3
- -	IgG4

GlyLeuTyrSerLeuSerSerValValThrValProSerSerSerLeuGlyThrGlnThr	IgG1
- AsnPhe - - - -	IgG2
- -	IgG3
- Lys -	IgG4

	Glm(17)	
TyrIleCysAsnValAsnHisLysProSerAsnThrLysValAspLysLysVal		IgG1
- Thr - - - Asp - - - - - - - - - - - - - - - Thr -		IgG2
- Thr - Arg -		IgG3
- Thr - - - Asp - - - - - - - - - - - - - - - - Arg -		IgG4

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*Fig. 4b.*Human immunoglobulin sequences hinge region

GluProLys	SerCysAspLysThrHisThrCysProPro	IgG1
GlxArgLys	CysCys Val Glx CysProPro	IgG2
GluLeuLysThrProLeuGlyAspThrThrHisThrCysProArgCysProGlu		IgG3
GluSerLysTyrGly	ProProCysProPro	IgG4
		IgG1
		IgG2
ProLysSerCysAspThrProProProCysProArgCysProGluProLysSer		IgG3
		IgG4
		IgG1
		IgG2
CysAspThrProProProCysProArgCysProGluProLysSerCysAspThr		IgG3
		IgG4
	CysPro	IgG1
	CysPro	IgG2
ProProProCysProArgCysPro		IgG3
	CysPro	IgG4



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Fig. 4c.

Human immunoglobulin sequences CH2 region

AlaProGluLeuLeuGlyGly	IgG1
- - ProValAla -	IgG2
- - - - -	IgG3
- - - Phe - - -	IgG4

ProSerValPheLeuPheProProLysProLysAspThrLeuMetIleSerArgThrPro	IgG1
- - - - -	IgG2
- - - - -	IgG3
- - - - -	IgG4

GluValThrCysValValValAspValSerHisGluAspProGluValLysPheAsnTrp	IgG1
- - - - - Gln - - -	IgG2
- - - - - Gln - Lys -	IgG3
- - - - - Gln - - - Gln - - -	IgG4

TyrValAspGlyValGluValHisAsnAlaLysThrLysProArgGluGluGlnTyrAsn	IgG1
- - - - - Phe -	IgG2
- - - - -	IgG3
- - - - - Phe -	IgG4

SerThrTyrArgValValSerValLeuThrValLeuHisGlnAspTrpLeuAsnGlyLys	IgG1
- - Phe - - - - Val - - - -	IgG2
- - Phe - - - -	IgG3
- - - - -	IgG4

GluTyrLysCysLysValSerAsnLysAlaLeuProAlaProIleGluLysThrIleSer	IgG1
- - - - - Gly - - - -	IgG2
- - - - -	IgG3
- - - - - Gly - - SerSer - - - -	IgG4

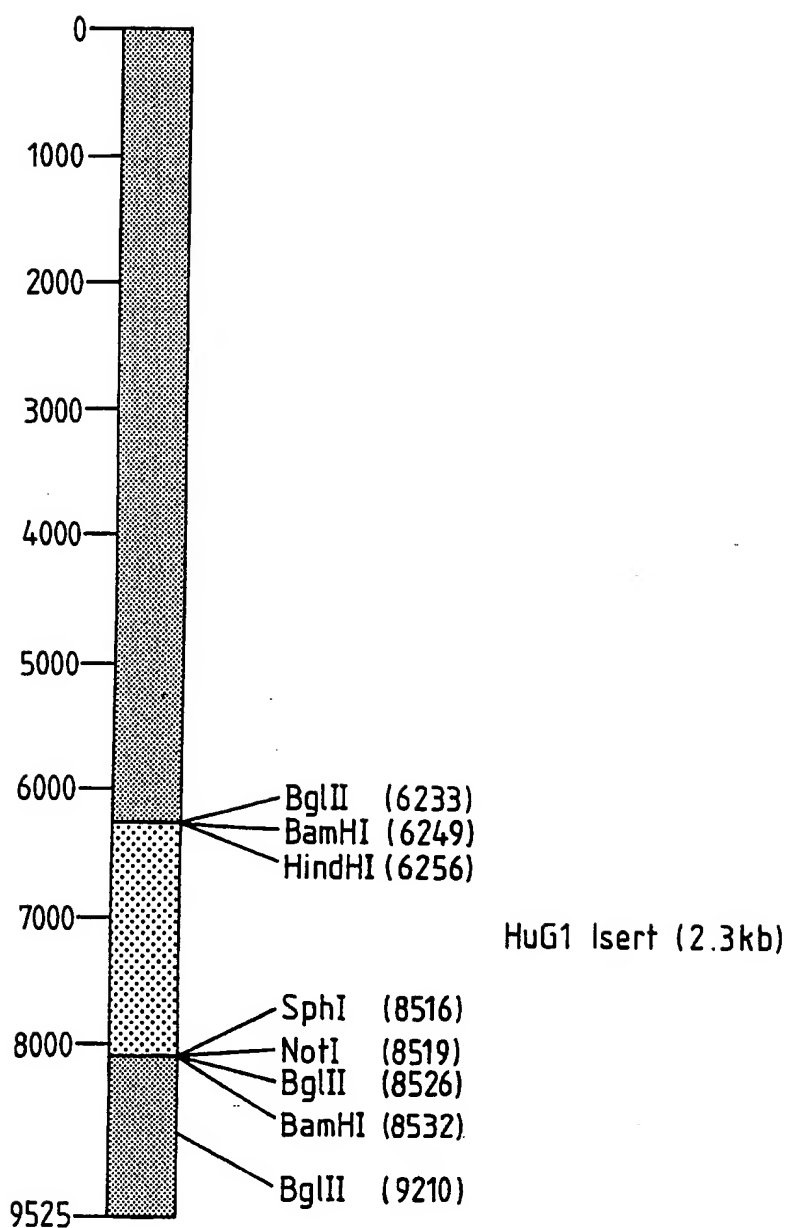
LysAlaLys	IgG1
- Thr -	IgG2
- Thr -	IgG3
- - -	IgG4

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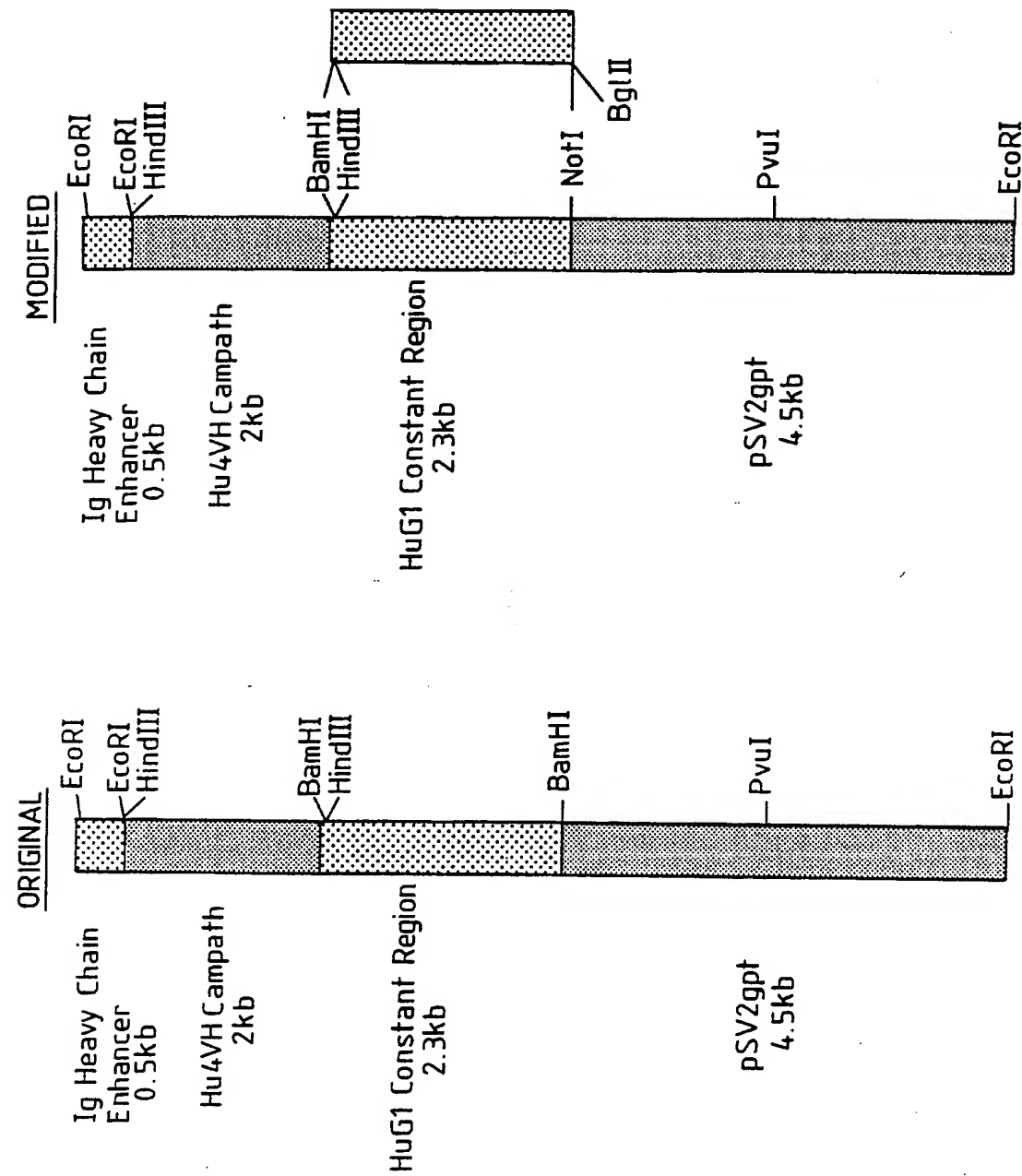
*Fig. 4d.*Human immunoglobulin sequences CH3 region

																	Glm(1)
Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Asp	Glu	IgG1
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Glu	IgG2
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Glu	IgG3
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	GlnGlu	IgG4
																	Glm(1)
Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	IgG1
Met	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	IgG2
Met	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	IgG3
Met	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	IgG4
																	Glm(1)
Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	IgG1
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	IgG2
-	-	-	-	-	-	Ser	-	-	-	-	-	-	Asn	-	-	-	IgG3
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	IgG4
																	Glm(1)
Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	IgG1
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	IgG2
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	IgG3
-	-	-	-	-	-	-	-	-	-	Arg	-	-	-	-	-	-	IgG4
																	Glm(2)
Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Gly	Leu	His	Asn	IgG1
-	-	-	-	-	-	-	-	-	-	-	-	-	Ala	-	-	-	IgG2
-	-	-	-	Ile	-	-	-	-	-	-	-	-	Ala	-	-	ArgPhe	IgG3
-	-	-	-	-	-	-	-	-	-	-	-	-	Ala	-	-	-	IgG4
																	Glm(2)
Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Lys								IgG1
-	-	-	-	-	-	-	-	-	-								IgG2
-	-	-	-	-	-	-	-	-	-								IgG3
-	-	-	-	-	-	-	Leu	-	-								IgG4

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*Fig. 5.*

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Fig. 7.

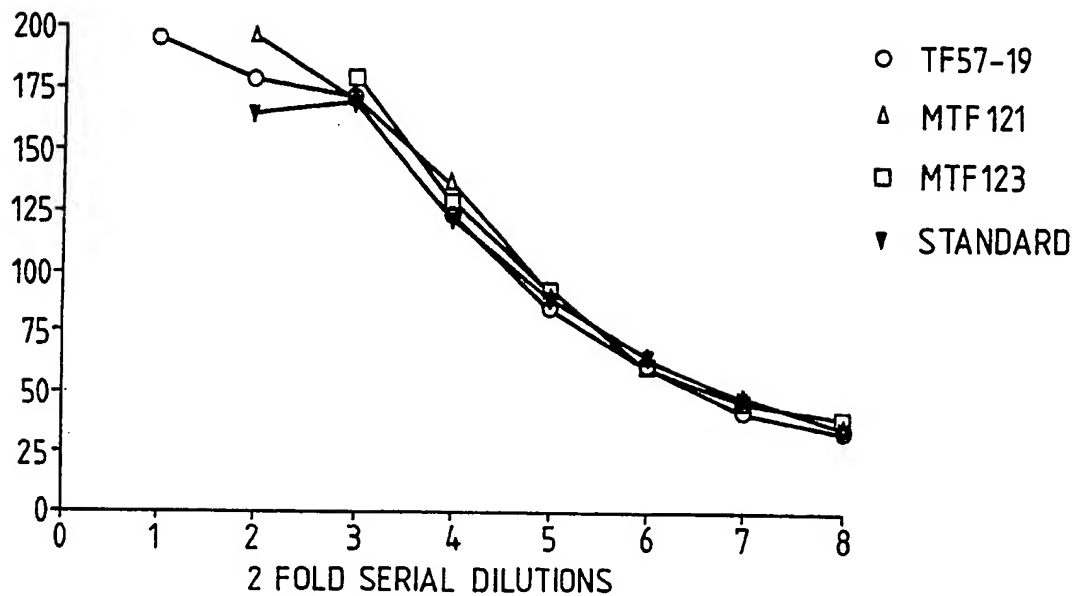
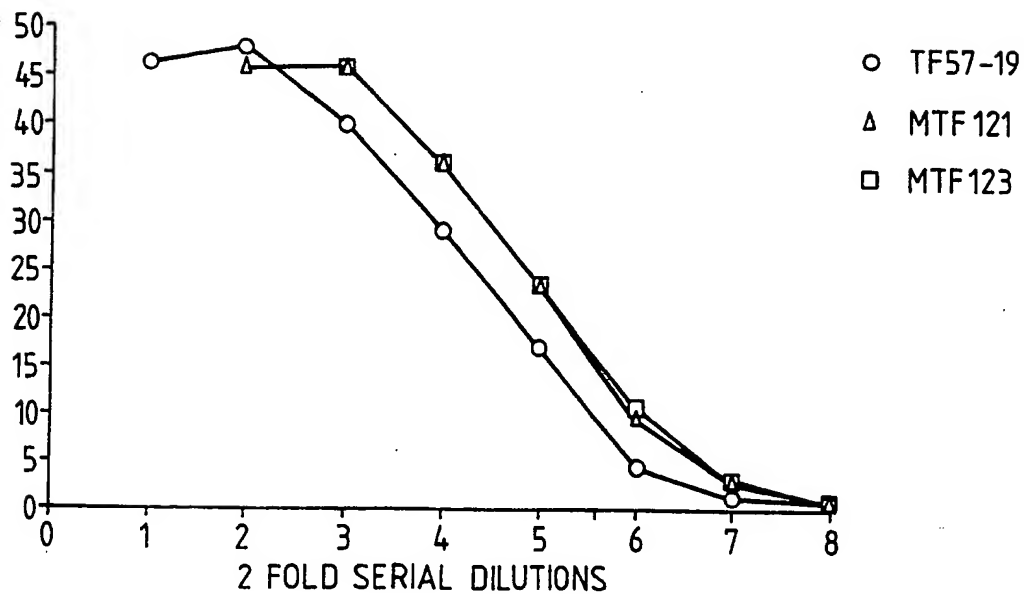
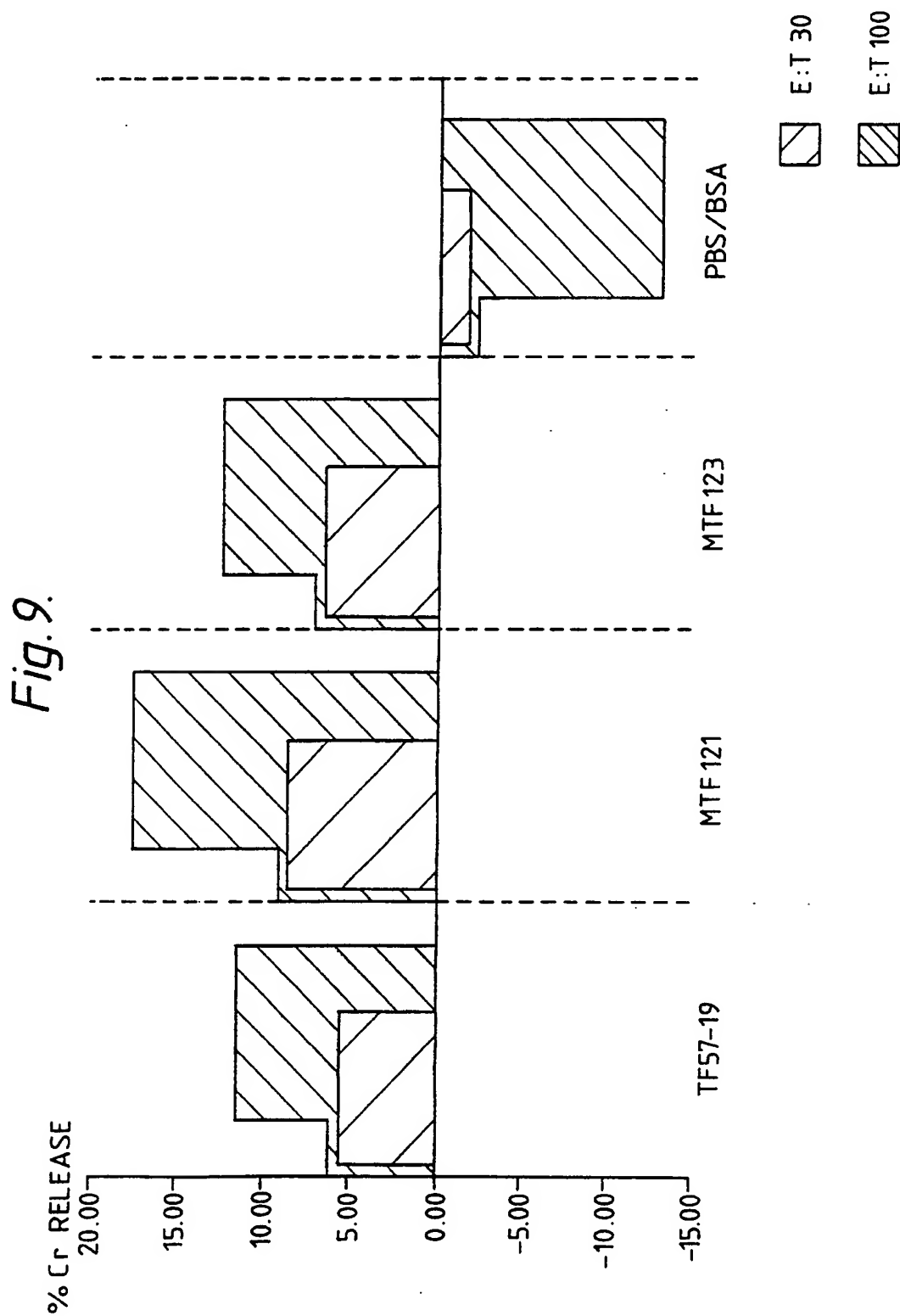


Fig. 8.



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## PCT/GB 92/00445

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III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	EP,A,0328404 (MEDICAL RESEARCH COUNCIL) 16 August 1989, see the whole document ---	1-19
A	W.E. PAUL, M.D.: "Fundamental Immunology", 1984, Raven Press, New York, US; chapter 9: J.B. FLEICHMAN et al.: "Immunoglobulins: Allotypes and Idotypes", see the whole document -----	1-19



## FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☒ OBSERVATION WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE <sup>1</sup>

This International search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claim numbers \_\_\_\_\_ because they relate to subject matter not required to be searched by this Authority, namely:  
Although claim 19 is directed to a method of the human body the search has been carried out and based on the alleged effects of the composition.
2. ☐ Claim numbers \_\_\_\_\_ because they relate to parts of the International application that do not comply with the prescribed requirements to such an extent that no meaningful International search can be carried out, specifically:
3. ☐ Claim numbers \_\_\_\_\_ because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING <sup>2</sup>

This International Searching Authority found multiple inventions in this International application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International search report covers all searchable claims of the International application.
2. ☐ As only some of the required additional search fees were timely paid by the applicant, this International search report covers only those claims of the International application for which fees were paid, specifically claims:
3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:
4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

## Remark on Protest

- ☐ The additional search fees were accompanied by applicant's protest.  
☐ No protest accompanied the payment of additional search fees.

SA 57491

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82

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